Neural mechanisms generating locomotion studied in mammalian brain stem–spinal cord in vitro

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ABSTRACT

The neural control system for generation of locomotion is an important system for analysis of neural mechanisms underlying complex motor acts. In these studies, a novel experimental model using neonatal rat brain stem and spinal cord in vitro was developed for investigation of the locomotor system in mammals. The in vitro brain stem and spinal cord system was shown to retain functional circuitry for locomotor command generation, motor pattern generation, and sensorimotor integration. This system was exploited to investigate neurochemical mechanisms involved in neurogenesis of locomotion. Evidence was obtained for peptidergic and γ-aminobutyric acid-mediated mechanisms in brain-stem circuits generating locomotor commands. Cholinergic, dopaminergic, and excitatory amino acid-mediated mechanisms were shown to activate spinal cord circuits for locomotor pattern generation. Endogenous *N*-methyl-D-aspartic acid receptors in spinal networks were found to play a central role in the generation of locomotion. The chemically induced patterns of motor activity and rhythmic membrane potential oscillations of spinal motoneurons were characteristic of those during locomotion in other mammals in vivo. The in vitro brain stem and spinal cord model provides a versatile and powerful experimental system with potentially broad application for investigation of diverse aspects of the neurobiology of mammalian motor control systems. — SMITH, J. C.; FELDMAN, J. L.; SCHMIDT, B. J. Neural mechanisms generating locomotion studied in mammalian brain stem-spinal cord in vitro. *FASEB J.* 2: 2283-2288; 1988.

Key Words: neural pattern generation • motor control systems • excitatory amino acid neurotransmitters • N-methyl-D-aspartic acid receptors

THE NEUROGENESIS OF LOCOMOTION involves interaction of brain-stem networks for generation of motor commands, spinal cord motor pattern-generating networks, and sensorimotor circuits (1–4). Analysis of neuronal and network properties underlying the generation of locomotion and other rhythmic movements of brain stem and spinal cord origin is a necessary step toward understanding central nervous system (CNS) control of complex motor acts (3, 4). Identifying neuronal membrane and neurochemical mechanisms responsible for the signal processing and interneuronal communication that underlie these acts is fundamental. These mechanisms have not been identified (2), largely because of the difficulty in performing neurochemical and membrane biophysical studies in the mammalian CNS in vivo. As a step toward overcoming these limitations, we have recently developed in vitro preparations of the mammalian brain stem and spinal cord that provide a novel experimental system for investigation of motor control systems for rhythmic movements (5–8; see also ref 9). The in vitro system offers several important advantages over conventional in vivo approaches, including precise control of CNS extracellular environment and mechanical stability for long-term intracellular recording, that are necessary for detailed analysis of neural mechanisms.

In the work reported here, we have extended our earlier observations on viability of locomotor and other motor circuits in the in vitro brain stem–spinal cord (5–8) and have now established that this system retains functional circuitry for the major neural components of the locomotor control system, including circuits for motor command generaton, motor pattern generation, and sensorimotor integration. We exploited this system to identify endogenous neurochemical mechanisms involved in motor command and pattern generation. Our data indicate that diverse neurochemical mechanisms are involved, and that there is an essential role for excitatory amino acids acting at *N*-methyl-D-aspartic acid (NMDA) receptors in locomotor pattern generation. Similar findings have been obtained in lower vertebrates (3, 10–12), and our results with the mammalian system are consistent with the hypothesis (5, 13) that there is an evolutionary conservation of mechanisms for rhythmic locomotor pattern generation in vertebrates.

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RESULTS

Isolated brain stem and spinal cord from 0- to 4-day-old Sprague-Dawley rats were maintained under in vitro conditions[2] in an experimental recording chamber as previously described (7). The neonatal rat CNS has several properties that permit viability for extended periods in vitro.[3] Brain-stem or spinal locomotor circuitry could be repeatedly activated by selectively applying specific neurochemicals to the brain-stem or spinal cord bathing solution in preparations where the in vitro bath was partitioned at the spinomedullary junction with a barrier (7).[2] Stereotypical patterns of rhythmic locomotor activity in spinal ventral roots and individual motoneurons (Fig. 1) could be generated for periods of up to 8 h. The locomotor nature of the motor patterns was verified with preparations retaining pairs of innervated hindlimbs or forelimbs in vitro[2] (6–8; see also ref 16). The chemically evoked motoneuronal activity produced multijoint, alternating steppinglike movements of the limbs. Electromyographic (EMG) recordings from hindlimb muscle confirmed the presence of alternating patterns of flexor-extensor muscle activation characteristic of those during locomotor movements in intact quadrupeds. The patterns of activity of flexor-extensor muscles acting at the hip (sartorius, semimembranosus; Fig. 1*B*), knee (semitendinosus, rectus femorus), and ankle (tibialis anterior, gastrocnemius; see also ref 16) were similar to those described during quadruped walking (17). The patterns of limb motor nerve activity recorded extracellularly, as well as the rhythmic oscillations of motoneuron membrane potential recorded intracellularly (Fig. 1*C*), were also characteristic of quadruped walking (1, 14, 17). Patterns for other locomotor gaits such as galloping could be generated by neurochemical activation of brain-stem circuitry.

The chemically induced motor patterns resembled those elicited by activating spinal sensory afferent pathways. Pinching the tail or skin at the base of the tail in preparations retaining hindlimbs and tail, or direct electrical stimulation of lumbar dorsal roots, elicited transient bouts of motoneuronal activity for walking (Fig. 2). The central pattern-generating mechanism remained responsive to peripheral afferent inputs for periods of up to 7–8 h, indicating long-term viability of sensorimotor reflex pathways. The correspondence between the motor patterns evoked chemically and by physiological stimuli (e.g., tail pinch) that induce locomotion in vivo (1) suggests that the neurochemicals used to elicit locomotor output activated normal pattern generation mechanisms.

Stimulation of several locomotor command-generating regions in the brain stem in vivo can activate spinal locomotor networks via descending bulbospinal motor command pathways (1, 19, 20). We specifically tested neurochemicals likely to activate the mesencephalic locomotor region (MLR), a major command-generating area in the midbrain tegmentum receiving input from the motor cortex and basal ganglia (20).[4] With the brain stem transected in vitro at the midcollicular level to retain the MLR, the excitatory amino acid L-glutamic acid, the neuropeptide substance P, or antagonists to γ-aminobutyric acid (GABA) (bicuculline, picrotoxin) applied to the solution bathing the brain stem induced locomotor activity (Fig. 1, *D* and *E*). Threshold concentrations of GABA antagonists or substance P for induction of locomotor output were 1–10 μM. The onset of motor activity occurred within 5–10 min after bath application of the neurochemicals. The motor output induced by substance P frequently subsided after 15–30 min, probably owing to inactivation of the peptide in the CNS, whereas the motor activity induced by GABA antagonists continued for several hours. The motor activity typically consisted of recurring episodes of 10–30 cycles of rhythmic motor discharge with interlimb coordination patterns for walking (Fig. 1*D*), with initially high discharge frequencies and amplitudes that progressively declined as the bout of locomotor activity subsided. The episodes of rhythmic activity were separated by 20- to 60-s periods of low-level tonic motoneuronal activity where presumably spinal neuron excitation was insufficient to activate the motor pattern generation system. L-Glutamic acid (0.5–1 mM) administered with the amino acid uptake inhibitor dihydrokainic acid (DHK; 0.2 mM) produced either transient locomotor activity (n = 4), or recurring episodes (n = 5) similar to those induced by GABA antagonists. The average cycle durations recorded during rhythmic activity induced by bicuculline (5–10 μM) or picrotoxin (10 μM) [2.6 ± 0.6 (n = 7) and 2.5 ± 0.5 s (n = 2), respectively] were similar to cycle durations after activation with substance P (5 μM) (3.1 ± 0.6 s, n = 4) or L-glutamic acid (1 mM) (2.5 ± 0.4 s, n = 5); the lowest cycle periods in all cases were 1.2–1.4 s. These cycle periods are at the upper end of the range expected for intact quadrupeds during ambulation (1).[5]

The above findings are consistent with studies in vivo (19, 20) that suggest the presence of GABA-mediated inhibitory synaptic inputs, and substance P-mediated excitatory inputs to the MLR from basal ganglia output nuclei. There is evidence (20) that substance P may

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[2]The standard bathing solution contained 128 mM NaCl, 3.0 mM KCl, 0.5 mM NaH_2PO_4, 1.5 mM $CaCl_2$, 1.0 mM $MgSO_4$, 21 mM $NaHCO_3$, 30 mM glucose, equilibrated with 95% O_2-5% CO_2 (pH ≅ 7.4) at 27°C. The in vitro bath was partitioned at the spinomedullary junction with a vaseline barrier to isolate the bathing solutions for the brain stem and spinal cord, which allowed independent manipulation of the ionic or neurochemical concentrations in the brain-stem and spinal cord solutions. With preparations retaining limbs, the skin is removed from the limbs and muscle viability is maintained by the bathing medium.

[3]The small size of the neonatal rat brain stem and spinal cord with short diffusion distances allows maintenance of CNS viability by diffusion of O_2 and metabolic substrates from the bathing medium. The tolerance of mammalian neonatal CNS to hypoxia and anoxia further permits maintenance of neuronal energy metabolism and viability (7). Viability of brain stem–spinal cord circuits could be maintained in vitro in some preparations for periods of up to 8–12 h.

[4]Evidence suggests that the MLR corresponds in part to the pedunculopontine nucleus (PPN) (20). Developmental studies (21) have verified a well-defined PPN in neonatal rat brain stem.

[5]The longer locomotor cycle periods in vitro result in part from the lower CNS temperature.

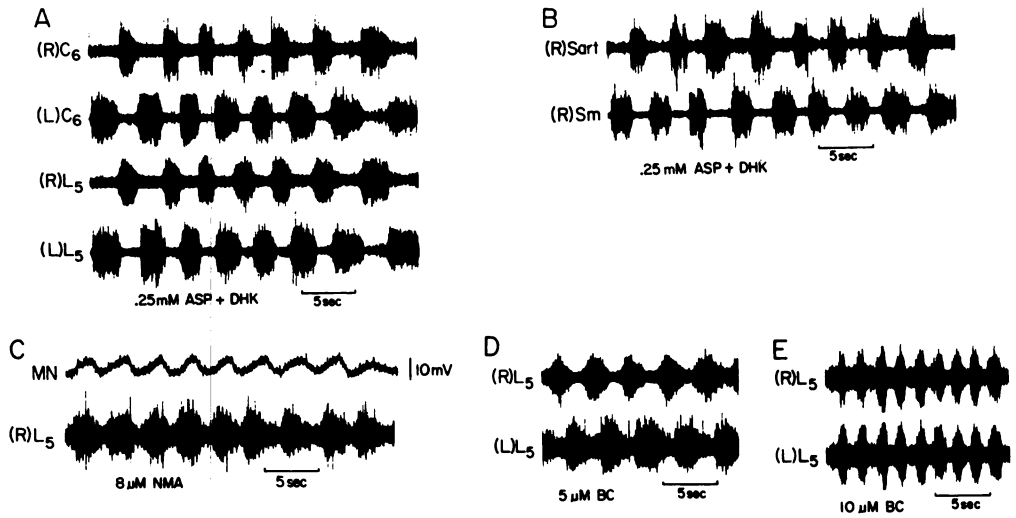

Figure 1. Patterns of motor activity generated in vitro by chemical activation of locomotor circuitry. *A*) Hindlimb and forelimb ventral root activity elicited by 0.25 mM L-aspartic acid (ASP) with 0.2 mM dihydrokainic acid (DHK) in spinal cord bath (see Fig. 3), illustrating a general pattern of ventral root activity induced by chemical activation of spinal circuitry. Recordings were obtained with suction electrodes on left (L) and right (R) ventral roots at the sixth cervical (C_6) and fifth lumbar (L_5) segment levels. Motor output on a given ventral root consists of rhythmic discharge with two phases corresponding to limb flexion and extension. For each trace shown, the ventral root discharge correlated mainly with flexor muscle activity as determined by limb electromyograms (EMG); extensor activity could be recorded (see *B*) on other ventral roots. Phase lag of 50% of the cycle period (i.e., 180° phase difference) occurs between ipsi- and contralateral ventral root flexor (or extensor) activity recorded at the same segmental level for all cycle frequencies. The phase lag between ipsilateral hindlimb and forelimb flexor (or extensor) activity was less than 10% of cycle period. These temporal patterns are characteristic of one type of quadruped walking (1). Motor patterns for other gaits such as galloping where hindlimb movements are synchronous, alternating with synchronous forelimb movements, were generated by activation of brain-stem locomotor regions (see *E*). *B*) Recordings of hindlimb EMGs during limb movements induced by activating spinal circuitry with L-aspartic acid and DHK. Traces show activity of hip flexor muscle [sartorius (Sart)] and hip extensor muscle [semimembranosus (Sm)] with alternating pattern of flexor-extensor activation characteristic of quadruped locomotion. *C*) L_5 ventral root recording and intracellular recording of activity of ipsilateral motoneuron (nonspiking) in L_5 segment showing rhythmic locomotor drive potentials (LDPs) in motoneuron (MN) during locomotion induced by *N*-methyl-DL-aspartic acid (NMA) applied to spinal cord. Motoneuron resting membrane potential was −52 mV. Intracellular recording was obtained with a microelectrode filled with 3 M potassium acetate. LDPs like those recorded in vitro have been described in cat motoneurons (14), as well as in lower vertebrates (15), and are believed to reflect alternating excitatory and inhibitory drive to motoneurons (14). *D, E*) Examples of hindlimb ventral root discharge patterns with activation of brain-stem circuitry by γ-aminobutyric acid (GABA) antagonist bicuculline (BC) showing alternating motor patterns for walking (*D*) and synchronous discharge pattern for galloping (*E*) generated in preparations retaining the mesencephalic locomotor region. Average cycle frequency with galloping pattern was 1.0–0.8 Hz. A continuous sequence of motor activity starting with motor patterns for walking followed by patterns for trotting and then galloping could be generated by blocking GABAergic inhibition in some preparations. The pattern of activity evoked by GABA antagonists was dose-dependent; motor patterns as shown were typically induced with bicuculline concentrations < 20 μM. Substance P or L-glutamic acid induced walking patterns similar to those in *D*.

also mediate excitatory connections between the MLR and neurons in the ventromedial medullary reticular formation, a proposed relay for descending transmission of locomotor commands. Consistent with this hypothesis, substance P also induced motor patterns for walking in vitro with the brainstem transected at the pontomedullary junction, excluding the MLR, although locomotor cycle durations (8.9 ± 2.6 s with 10 μM substance P, $n = 4$) were typically longer than in preparations retaining the MLR. Locomotor command generation at the supraspinal level probably involves a complex system of excitatory and inhibitory connections among the basal ganglia, MLR, and medullary reticular formation (20). From our results and previous in vivo studies (19, 20), GABAergic inhibition appears to play a major role in this system. Removal of this inhibition in vivo is sufficient to activate circuitry that generates movement synergies ranging from walking and trotting to galloping. Blocking GABAergic inhibition in vitro similarly induced motor patterns for several locomotor gaits (Fig. 1, *D* and *E*), confirming the presence of functional circuitry for generation of command signals for various movement synergies.

The locomotor circuitry in the spinal cord could be directly activated by certain excitatory amino acids, dopaminergic agonists, or cholinergic agonists applied to the spinal cord bath. The latency to onset of motor activity after bath application of the agonists was 1–5 min. Dopamine-induced activity was suppressed by the D_2 receptor antagonists haloperidol (50–100 μM). Anticholinesterase (25–50 μM edrophonium) was required with acetylcholine (20–50 μM) to generate continuous locomotor activity. Activity induced by acetylcholine or edrophonium was suppressed by the muscarinic receptor antagonist atropine sulfate (10–50 μM). The locomotor cycle period decreased with increasing neurochemical concentration. The cycle period decreased from 15.5 ± 3.7 to 4.0 ± 0.5 s ($n = 4$) over the effective dopamine concentration range (0.1–0.7 mM); it decreased similarly from 13.5 ± 3.5 to 3.5 ± 0.6 s ($n = 4$) over the effective acetylcholine concentration range (10–50 μM in the presence of 50 μM edropho-

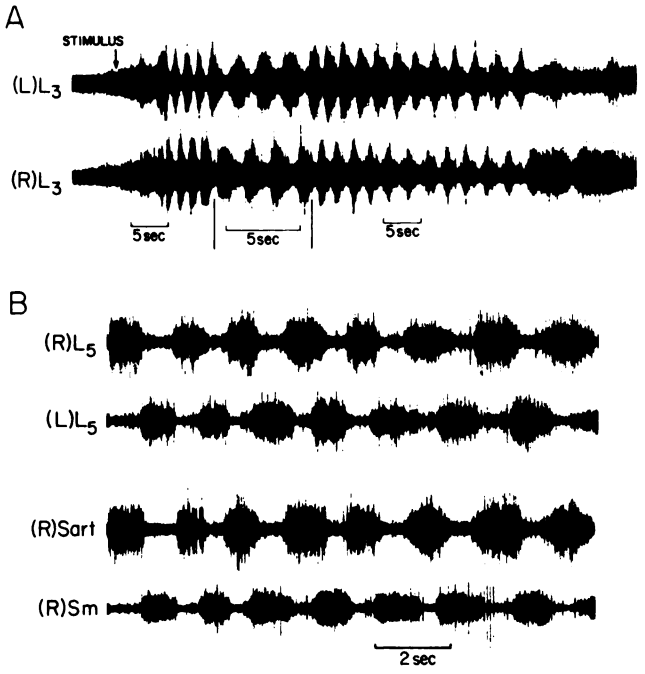

Figure 2. Locomotor pattern generated by stimulating somatosensory afferent pathways by pinching base of tail in spinal cord preparation retaining limbs and tail (*A* and *B*). Short duration (~1 s) tail pinch induces transient bouts of hindlimb-forelimb locomotor activity consisting of 5–30 locomotor cycles as illustrated in *A* by recordings of motor discharge on third lumbar (L_3) ventral roots. Average cycle period during locomotor episodes was typically 1.5–2.5 s. Similar motor patterns were elicited by electrical stimulation (0.2-ms pulses, 1–50 Hz, 10–200 μA, 5- to 30-s train duration) of single lumbar dorsal roots. *B*) Patterns of ventral root discharge (top traces) and hindlimb EMG (bottom traces) induced by sensory activation are similar to walking patterns generated by chemical stimulation (Fig. 1). Rhythmic motor activity evoked by either sensory or chemical stimulation was suppressed by blocking spinal NMDA receptors, suggesting involvement of similar pattern generation mechanisms.

nium). Cycle frequency vs. agonist concentration relationships for excitatory amino acids are shown in Fig. 3. The motor output produced alternating steppinglike movements of the hindlimbs at all cycle speeds with typically an increase in the amplitude of the angular displacements of the limb joints as the level of excitation and cycle frequency increased. The cycle frequencies at the highest neurochemical concentrations overlap those with brain stem and sensory stimulation, implying similar levels of spinal neuronal excitation.

Evidence was obtained that these neurochemicals induced motor activity by mimicking the action of related endogenous neurochemicals on spinal neurons affecting locomotor pattern generation. Nonselective neuronal excitation was unlikely, because generalized depolarization of spinal neurons produced by elevating extracellular K^+ concentration from 3 to 7 mM, enough to evoke motoneuronal discharge, did not induce locomotor activity. To test for involvement of related endogenous neurochemicals, we probed the system with neurochemical uptake or breakdown inhibitors. The excitatory amino acid uptake inhibitor DHK (0.2–1 mM) (Fig. 3) or anticholinesterase (50–200 μM edrophonium) applied to the spinal cord alone elicited motor patterns for walking in a dose-dependent manner, indicating ongoing endogenous release of excitatory amino acids and acetylcholine whose synaptic concentrations can be brought to threshold for activation of the locomotor circuitry.

Earlier studies have shown that endogenous excitatory amino acids activating NMDA receptors[6] are involved in locomotor pattern generation in the spinal cord of lower vertebrates (2, 10–12). There is also evidence that NMDA receptor activation mediates polysynaptic excitation in spinal sensorimotor pathways (18). We therefore characterized the receptor subtypes mediating the action of the excitatory amino acids. NMDA-receptor agonists (NMDA, L-aspartic acid, and L-glutamic acid) most effectively induced locomotor activity; other excitatory amino acids (kainic and quisqualic acids) were weakly effective or ineffective.[6] The motor activity elicited by any of the amino acids or their uptake inhibitors was competitively inhibited by the selective NMDA-receptor antagonist DL-2-amino-5-phosphonovaleric acid (AP5) (Fig. 3). The involvement of NMDA receptors was further supported by the observations that: *1*) an elevated spinal extracellular Mg^{2+} concentration (10 mM) blocked locomotor activation by exogenous excitatory amino acids,[6] and *2*) AP5 (10–100 μM) applied to the spinal cord bath suppressed the rhythmic locomotor activity generated by activating brain-stem circuitry or sensory stimulation (6, 8), or by dopaminergic or cholinergic agonists. Therefore, all locomotor activity ultimately depended on activation of spinal NMDA receptors.

DISCUSSION

The results of this work establish the feasibility of investigating diverse aspects of the locomotor control system in vitro including motor command and pattern generation, as well as mechanisms for sensorimotor integration. In vitro brain stem and spinal cord are already routinely used to investigate motor systems in lower vertebrates (2, 3, 22–24), and there have been efforts recently to develop similar in vitro mammalian preparations (5–8, 9, 16, 25–29). The in vitro system developed in these studies is the first mammalian system shown to retain functional components of the brain stem–spinal cord locomotor control circuitry. This system also retains functional circuitry for other rhythmic movements of brain stem–spinal cord origin (respiratory movements)

[6]Three classes of excitatory amino acid receptors named after the selective agonists NMDA, kainic acid, and quisqualic acid have been proposed to mediate the actions of excitatory amino acid neurotransmitters in the vertebrate CNS (18). NMDA receptor agonists are potent activators of the spinal locomotor circuitry, whereas quisqualic acid (0.1–100 μM) applied to the spinal cord ($n = 4$) produced only unpatterned motoneuron discharge. Kainic acid (0.75–5 μM) induced rhythmic motoneuron discharge in three of six preparations tested; selective kainic acid receptor antagonists are not available and pharmacological specificity could not be confirmed. NMDA receptors are potently blocked by Mg^{2+} elevated above physiological concentrations of ~1 mM (18).

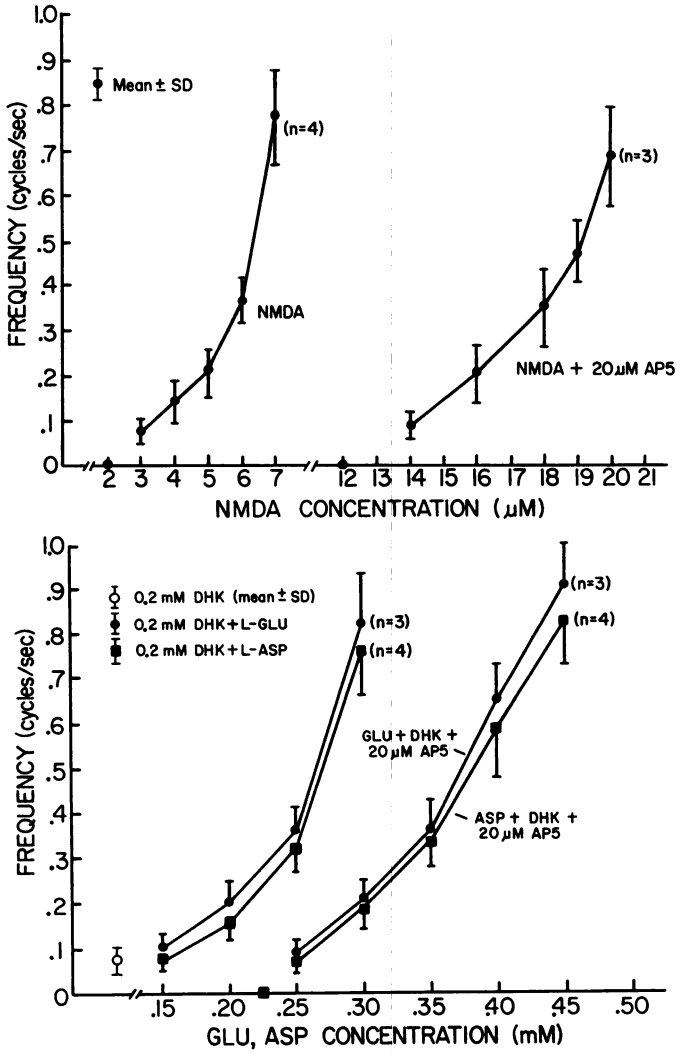

Figure 3. Involvement of excitatory amino acids in locomotor pattern generation. NMDA, L-aspartic acid (L-ASP), and L-glutamic acid (L-GLU) applied to spinal cord bath induce rhythmic motoneuronal discharge; discharge frequency increases with agonist concentration to a maximum value beyond which the discharge becomes tonic. NMDA alone induces continuous locomotor activity lasting for several hours; threshold concentration for NMDA is ~3-4 μM with cycle frequency increasing over a narrow concentration range (~3-7 μM). Discharge frequency vs. agonist concentration relationships for L-ASP and L-GLU were obtained with amino acid uptake inhibitor DHK (0.2 mM) in the bathing solution; without DHK, L-GLU and L-ASP induce only transient episodes of rhythmic motoneuronal discharge. After a 15-min pretreatment of the spinal cord with DHK, the motor discharge is steady for periods of up to several hours. DHK (0.2 mM) alone induces low-frequency rhythmic discharge that is suppressed by NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (AP5); higher discharge frequencies (not shown) can be induced by DHK in a dose-dependent manner (0.2-1 mM). Threshold concentrations for effects of L-GLU and L-ASP on cycle frequency in the presence of DHK is ~150 μM. Amino acid dose-response relationships are displaced by AP5, indicating competitive antagonism and involvement of NMDA receptors in rhythm generation.

(5, 7, 9), and it therefore appears that it has potentially broad application for investigation of major motor control systems. Our demonstration of the viability of motor system circuits in vitro should encourage further investigation with this system into locomotor networks and other mechanisms involved in the neurogenesis of movement.

Our results suggest that diverse endogenous neurochemical mechanisms underlie the generation of locomotion, including peptidergic and GABAergic mechanisms in brain-stem networks, and dopaminergic, cholinergic, and excitatory amino acid-mediated mechanisms in spinal cord networks. This diversity undoubtedly reflects the multiple interacting networks of the motor command and pattern generation systems. The neuronal interconnections and neurotransmitters in these networks are unknown. The present results provide evidence for several important endogenous neurochemical mechanisms. The spinal networks receive multiple inputs from bulbospinal and sensory afferent systems that may converge on a common set of spinal interneurons critical for locomotor pattern generation (1). Our results are consistent with this hypothesis. Locomotor activity induced by sensory or brain-stem inputs or by dopaminergic or cholinergic receptor activation was suppressed by blocking NMDA receptors in the spinal cord. These results suggest the following possibilities. *1*) Bulbospinal, sensory afferent, and dopamine-activated and acetylcholine-activated pathways all release excitatory amino acids that activate a common set of interneurons critical for locomotor pattern generation. If true, this means that the descending bulbospinal locomotor command signals are transmitted by excitatory amino acids activating NMDA receptors. *2*) Activation of the spinal locomotor circuitry requires tonic (permissive) inputs from interneurons releasing excitatory amino acids that activate NMDA receptors.

Our findings of a central role for endogenous excitatory amino acids activating NMDA receptors in neurogenesis of locomotion extend similar findings for lower vertebrates. Rhythmic locomotor movements ranging from undulatory swimming in primitive cyclostomes (2, 3) to walking by tetrapod mammals now appear to depend on this excitatory amino acid-mediated mechanism, which suggests a phylogenetic conservation of this mechanism for rhythmic motor pattern generation (5, 13). These locomotor pattern generation systems consist, in part, of oscillation-generating circuitry producing the locomotor cycle rhythm (1-4). The data indicate that excitatory amino acids are important for activating this component (Fig. 3). Inasmuch as NMDA-receptor activation can induce bistable neuronal membrane properties, these receptors may also be directly involved in the generation of oscillatory activity (15). However, the neurons activated by the excitatory amino acids and the relation between their activity and rhythmogenesis have not been determined in any vertebrate system. The in vitro system developed here should provide the opportunity to determine the underlying neuronal mechanisms in the mammalian system. FJ

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REFERENCES

1. GRILLNER, S. Control of locomotion in bipeds, tetrapods, and fish. Brookhart, J. M.; Mountcastle, V. B.; Kandel, E. R., eds. *Handbook of physiology, Section 1, The nervous system, Vol. II, Motor control, Part 2.* Bethesda: American Physiological Society; 1981: 1179–1236.
2. GRILLNER, S.; WALLÉN, P. Central pattern generators for locomotion, with special reference to vertebrates. *Annu. Rev. Neurosci.* 8: 233–261; 1985.
3. GRILLNER, S. Neurobiological bases of rhythmic motor acts. *Science* 228: 143–149; 1985.
4. FELDMAN, J. L.; GRILLNER, S. Control of vertebrate respiration and locomotion: a brief account. *Physiologist* 26: 310–316; 1983.
5. SMITH, J. C.; FELDMAN, J. L. Motor patterns for respiration and locomotion generated by an in vitro brainstem-spinal cord from neonatal rat. *Soc. Neurosci. Abstr.* 11: 24; 1985.
6. SMITH, J. C.; GARCIA-RILL, E.; FELDMAN, J. L. Chemical activation of mammalian locomotion in vitro. *Soc. Neurosci. Abstr.* 12: 386; 1986.
7. SMITH, J. C.; FELDMAN, J. L. In vitro brainstem-spinal cord preparations for study of motor systems for mammalian respiration and locomotion. *J. Neurosci. Methods* 21: 321–333; 1987.
8. SMITH, J. C.; FELDMAN, J. L. Neural control system for mammalian locomotion in vitro. *Soc. Neurosci. Abstr.* 13: 825; 1987.
9. SUZUE, T. Respiratory rhythm generation in the in vitro brain stem-spinal cord preparation of the neonatal rat. *J. Physiol. (London)* 354: 173–183; 1984.
10. BRODIN, L.; GRILLNER, S. The role of putative excitatory amino acid neurotransmitters in the initiation of locomotion in the lamprey spinal cord. I. The effects of excitatory amino acid antagonists. *Brain Res.* 360: 139–148; 1985.
11. BRODIN, L.; GRILLNER, S. The role of putative excitatory amino acid neurotransmitters in the initiation of locomotion in the lamprey spinal cord. II. The effects of amino acid uptake inhibitors. *Brain Res.* 360: 149–158; 1985.
12. DALE, N.; ROBERTS, A. Excitatory amino-acid receptors in *Xenopus* embryo spinal cord and their role in the activation of swimming. *J. Physiol. (London)* 348: 527–543; 1984.
13. DALE, N. Excitatory synaptic drive for swimming mediated by amino acid receptors in the lamprey. *J. Neurosci.* 6: 2662–2675; 1986.
14. JORDAN, L. M. Factors determining motoneuron rhythmicity during fictive locomotion. *Symp. Soc. Exp. Biol.* 37: 423–444; 1983.
15. WALLÉN, P.; GRILLNER, S. *N*-Methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. *J. Neurosci.* 7: 2745–2755; 1987.
16. KUDO, N.; YAMADA, T. *N*-Methyl-D,L-aspartate induced locomotor activity in a spinal cord-hindlimb muscles preparation of the newborn rat studied in vitro. *Neurosci. Lett.* 75: 43–48; 1987.
17. PERRET, C. Centrally generated patterns of motoneuron activity during locomotion in the cat. *Symp. Soc. Exp. Biol.* 37: 405–422; 1983.
18. MAYER, M.; WESTBROOK, G. The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog. Neurobiol.* 28: 197–276; 1987.
19. GARCIA-RILL, E.; SKINNER, R. D.; FITZGERALD, J. A. Chemical activation of the mesencephalic locomotor region. *Brain Res.* 330: 43–54; 1985.
20. GARCIA-RILL, E. The basal ganglia and the locomotor regions. *Brain Res. Rev.* 11: 47–63; 1986.
21. CONRAD, C.; HENDERSON, V.; SKINNER, R. D.; ABRAHAM, P.; GARCIA-RILL, E. Development of the pedunculopontine nucleus. I. In vivo. *Soc. Neurosci. Abstr.* 13: 1176; 1987.
22. GRILLNER, S.; WALLÉN, P.; MCCLELLAN, A.; SIGVARDT, K.; WILLIAMS, T.; FELDMAN, J. L. The neural generation of locomotion in the lamprey: an incomplete account. *Symp. Soc. Exp. Biol.* 37: 285–303; 1983.
23. ROVAINEN, C. M. Generation of respiratory activity by the lamprey brain exposed to picrotoxin and strychnine, and weak synaptic inhibition in motoneurons. *Neuroscience* 10: 875–882; 1983.
24. MCCLELLAN, A. D. Descending control and sensory gating of "fictive" swimming and turning responses elicited in an in vitro brainstem/spinal cord preparation of the lamprey. *Brain Res.* 302: 151–162; 1984.
25. LLINÁS, R.; YAROM, Y.; SUGIMORI, M. Isolated mammalian brain in vitro: new technique for analysis of electrical activity of neuronal circuit function. *Federation Proc.* 40: 2240–2245; 1981.
26. BAGUST, J.; KELLEY, M. E. M.; KERKUT, G. A. An isolated mammalian brainstem-spinal cord preparation suitable for the investigation of descending control of motor activity. *Brain Res.* 327: 370–374; 1985.
27. RICHERSON, G. B.; GETTING, P. A. Respiratory activity in a perfused guinea pig brain/spinal cord preparation. *Soc. Neurosci. Abstr.* 10: 745; 1984.
28. SHAPOVALOV, A. I.; SHIRIAEV, B. I.; TAMAROVA, Z. A. A study of neuronal activity of mammalian superfused or intra-arterially perfused CNS preparations. Kerkut, G. A.; Wheal, H. V., eds. *Electrophysiology of isolated mammalian CNS preparations.* New York: Academic; 1981: 376–394.
29. WALTON, K.; LLINÁS, R. An isolated in vitro preparation of the neonatal rat brainstem and spinal cord. *Soc. Neurosci. Abstr.* 11: 24; 1985.

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